

Research Article**ISOLATION AND CHARACTERIZATION OF BINDING AGENT FROM BLACK GRAM****Abhishek Soni, Ojasvi Gupta *, Hemant Kumar Verma, Dr. Amit Chaudhary***School of Pharmacy, Abhilashi University, Chail Chowk, Mandi (H.P), INDIA.***Received on: 28-04-2019; Revised and Accepted on: 09-06-2019****ABSTRACT**

Development of new excipients is time consuming involves tedious procedures and highly expensive. Instead, identification of new uses for the existing substances is relatively and less time consuming. The intention of present study was designed for isolation and characterization of binding agent from the seeds of Vigna mungo (Black gram seeds) and explores its use as pharmaceutical excipients. Binding agent was isolated by using two different methods. The isolated binding agent (powder) was investigated for purity by carrying out chemical tests for different Phytochemical constituents and only carbohydrates were found to be present. The binding agent (powder) was further characterized for physical and flow properties. Powder has good swelling index 60, PH 6.2, Melting point 137, Moisture absorption 2.45, Loss on drying 1.25. The binding agent (powder) had good flow property as Carr's Index 17.30, Angle of repose 35.6 and Hausnor's ratio 1.21. From the study, it indicates that Vigna mungo seeds (Black gram seeds) powder has satisfactory PH and physicochemical Properties, which can be used as pharmaceutical excipients in formulating various dosage form.

KEYWORDS: Excipients, Powder, Vigna mungo, Seeds, Methods.**INTRODUCTION**

Excipients are defined as 'the substance used as a medium for giving a medicament ^[1]. The role of excipients in determining the quality of a formulation and in many cases the bioavailability of drug from tablets has received considerable attention. Binders are added to tablet formulation to impart plasticity and thus increase the interparticulate bonding strength within the tablet. The development of new excipients for potential use as binding agent in tablet formulations continues to be of interest. This is because different binding agents can be useful in achieving various tablet mechanical strength and drug release properties for different pharmaceutical purpose ^[2]. Several pharmaceutical excipients of plant origin, like starch, agar, alginates, carrageenan, guar gum, xanthan gum, gelatin, pectin, acacia, tragacanth, and cellulose find applications in the pharmaceutical industry as binding agents, disintegrates, sustaining agents, protective's, colloids, thickening agents, gelling agents, bases in suppositories, stabilizers, and coating materials ^[3].

Pharmaceutical Excipient:

Pharmaceutical excipients can be defined as nonactive

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ingredients that are mixed with therapeutically active compound(s) to form medicines. The ingredient which is not an active compound is regarded as excipients. Excipients affect the behavior and effectiveness of the drug product more and more functionality and significantly. The variability of active compounds, excipients and process are obvious components for the product variability.

Classification of Excipients:

Excipients are commonly classified according to their application and function in the drug products.

- Binders
- Diluents
- Lubricants, Glidants, Disintegrants
- Polishing Film formers and coatings agents
- Plasticizers, Colouring
- Suspending agents Preservatives, antioxidants
- Flavorings, Sweeteners, Taste improving agents
- Printing inks, Dispersing agents Gum ^[4]

Binders:

Binders are agents employed to impart cohesiveness to the granules. This ensures the tablet remains intact after compression. The development of new excipients for potential use as binding agent in tablet formulations continues to be of interest. This is because different binding agents can be useful in achieving various tablet mechanical strength and drug release properties for different pharmaceutical purpose. Natural polysaccharides are widely used in the pharmaceutical and food industry as excipients and additives due to their low toxicity, biodegradable, availability and low cost. Natural binders like different starches, gums, mucilage's dried fruits possess binding

capacity as well as some other properties like disintegrants, filler, sustain release, and these natural polymers are much safer and economical than polymers like PVP. Different starches like rice, potato, maize, corn, wheat, tapioca starch and gums like ferula gummosa boiss, gum olibanum, beilschmiedia seed gum, okro gum, aegle marmelod gum, gum cordial, okra gum and cassia roxburghii seeds gum and plant fruit like date palm fruit and orange peel pectin shows good potency as a binding agent.

Gums are widely employed in the pharmacy as thickeners, suspending agents, emulsifying agents, binders and film formers. With the increase in demand for natural gums, it has been necessary to explore the newer sources of gums to meet the industrial demands. India, due to its geographical and environmental positioning has traditionally been a good source for such products among the Asian countries. There are reports about the successful use of *Ferula gummosa* Boiss, *Gum Olibanum*, *Beilschmiedia mannii* and *Aegle marmelos* fruit gum as binding agent [5].

Black Gram:

Black gram (*Vigna mungo*.L) is an important pulse crop occupying unique position in Indian agriculture. It is under cultivation in India in about 3.25 million hectares and its annual production is 1.45 million tons. Legumes are a rich source of antioxidants in treating various ailments like liver diseases, rheumatism, diabetes, heart diseases and infections in the central nervous system. The root nodule of black gram is said to be narcotic, diuretic and is used as remedy for aching bones, dropsy and cephalgia. Legume nitrogen fixation starts with the formation of a nodule. *Rhizobium* invades the root and multiplies within the cortex cells. The plant supplies necessary nutrients and energy for the bacteria. Organic manure contributes to soil fertility due to addition of organic matter and nutrients such as nitrogen that is trapped by bacteria in the soil. The addition of organic manures in soil enhanced the symbiotic relationship between phyto chemical compounds. The main areas of production are Madhya Pradesh, Uttar Pradesh, Maharashtra, West Bengal, Andhra Pradesh and Karnataka. Today, this crop is grown world wide as a pulse crop, India being top in production. It is one of the nutritious pulse crops, popularly known as urdbean. Economically, its seeds are highly nutritious with protein (25-26%), carbohydrates (60%), fat (1.5%), minerals, aminoacids and vitamins. It is an important short duration pulse crop and self pollinated grain legume grown in many parts of India. However, its yield is low compared to other grain legumes. In view of declining production of pulse crops, there is a dire need to develop high yielding cultivars through exploiting genetic phenomenon so called heterosis. This tool can raise the production potential of blackgram crop [6].

Table No. 1: Black Gram quick facts

Name	Black gram
Scientific name	<i>Vigna mungo</i>
Colors	Black or grey black or mottles
Shapes	Ovoid to rounded
Calories	706 Kcal/cup
Major nutrients	Copper (225.67%) Iron (195.88%) Isoleucine (159.33) Valine (138.78%) Manganese (137.43%)



Fig. 1: Black gram/(*Vigna mungo*. L) Seeds

History of Black Gram:

Black gram is originated in India where it was cultivated from ancient times. Black gram was introduced by Indian immigrants to other topical areas.

Benefits of Black Gram:

- Aids in diabetes
- Treats nervous disorders
- Good for hair problems
- Treats digestive disorder
- Heart health
- Relieve inflammation
- Bone health
- Increase energy
- Good for pregnant women
- Treatment for dandruff

Restrictions for Black Gram:

- It should not be taken by those who are easily predisposed to rheumatic disease and urinary calculi.
- It contains high concentration of oxalic acid, which is not safe for rheumatic patient.
- Pregnant women who are suffering from constipation should avoid black gram.
- It could promote uric acid levels in the body so people with gallstones, kidney stones, gout should avoid its consumption.

Traditional Uses:

- Roots are used for abscess, ostealgia and inflammations.
- Seeds are used to treat anorexia, dyspepsia, constipation, haemorrhoids and hepatopathy.
- Germinated black gram is helpful for mild diabetes.
- It eliminates toxins from the body and purifies the system.
- It is a great supplement for people with malnutrition.
- It is used to heal nervous disorders such as schizophrenia, weakness of memory, hysteria and nervous weakness.
- Apply the paste of black gram on hair for dandruffs and cure various hair problems.
- To treat headache due to sinus.

Method for Extraction:

Infusion: Fresh infusion is prepared by macerating the crude drug for a short period of time with cold or boiling water.

Separation of crude product and peel – In this process the infused product is rubbed with hands and then the peels and crude drugs are separated from each other.

Grinding: is a process of removing material by abrasive action of a revolving wheel on the surface of a work-piece in order to bring it to required shape and size.

Screening: Screening or sieving is the process of separating particles by size.

Maceration: In this process solid ingredients are placed in a stoppered container with the whole of the solvent and allowed to stand for a period of at least 3 days with frequent agitation.

Separation: A separation process is a method that converts a mixture or solution of chemical substance into two or more distinct product mixtures.

Drying: Drying is a mass transfer process consisting of removal of water or another solvent by evaporation from a solid, semi-solid, or liquid. This process is used as a final production step.

MATERIALS AND METHODS^[7, 8]

Isolation of Powder from Black Gram:

The 500g *Vigna mungo* seeds were soaked in distilled water for 1 day. Wash that soaks seeds with water 3-4 times. Separate the peels and crude product. Then grind that crude product into fine powder. Then macerate that product. Separate and collect that product in muslin cloth. The collected material was dried at room temperature. The dried powder was sieved in sieve no # 80. The resultant powder was stored in a desiccator and used for the present study.

1. Organoleptic Evaluation:

The Organoleptic evaluation refers to the evaluation of color, odour, shape, taste and special features which include touch and texture. The majority of information on the identity, purity and quality of the material can be drawn from these observations.

2. Physicochemical Characterization:

Barley extract was characterized by various tests of identification.

a) Identification of Starch: Barley extract was subjected to Molish test, Benedicts test, Iodine test

b) Determination of Purity: To determine the purity tests for alkaloids, carbohydrates, flavonoids, steroids, saponins, tannins and phenols were carried out.

Solubility:

Solubility was checked with different solvents such as water, hot water, acetone, ethanol, methanol, ether, chloroform.

Swelling Index:

Swelling index of powder was determined by using modified method reported. One gram of powder (#100 mesh passed) was accurately weighed and transferred to a 100 ml stopper measuring cylinder. The initial volume of the powder in the measuring cylinder was noted. The volume was made up to 100 ml mark with distilled water. The cylinder was stopper, shaken gently and set aside for 24 hours. The volume occupied by the gum sediment was noted after 24 hours. Swelling index (SI) is expressed as a percentage and calculated according to the following equation.

$$SI = \frac{[(\text{Final volume} - \text{Initial volume}) / \text{Initial volume}] \times 100}{100}$$

Moisture Absorption:

The Barley extract was weighed accurately and placed in a desiccator. After 3 days, the powder was taken out and weighed. The percentage of moisture uptake was calculated as the difference between final weight and initial weight with respect to initial weight.

pH of Starch:

The pH of 1% w/v dispersion of the powder was determined using a digital pH meter.

Melting Point:

The powdered sample of Barley was transferred into a capillary tube and by using melting point apparatus melting point was determined.

Loss on Drying (LOD):

Moisture content of Barley extract was determined by loss on drying method. Accurately weighed 1g sample was heated at 105°C to get a constant weight in a hot air oven and percent loss of moisture on drying was calculated using formula given below.

$$LOD (\%) = \frac{(\text{Weight of moisture in sample} / \text{Weight of sample before drying}) \times 100}{100}$$

Thermal Stability:

A sufficient quantity of the banana powder was taken in a petri dish and kept at successive higher temperatures (30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C, 100°C, 110°C, 120°C, 130°C and 140°C). The temperature at which the powder showed a change in color was noted.

Flow Properties of selected Polymers:

a) Bulk density:

Density is defined as mass per unit volume. Bulk density, pb is defined as the mass of the powder divided by the bulk volume and is expressed as g/cm. It depends upon particle size distribution, particle shape and the particles adhere together. Apparent bulk density (pb) was determined by pouring the blend into a graduated cylinder. The bulk density was calculated using the formula.

$$pb = M / Vb \dots (\text{eq.1})$$

Where, pb is bulk density, Vb is bulk volume, M is the weight of the powder.

b) Tapped density: The measuring cylinder containing a known mass of blend was tapped for a 100 times using density apparatus. The minimum volume (Vt) occupied in the cylinder and the weight (M) of the blend was measured. The tapped density (pt) was calculated using the formula.

$$pt = M / Vt \dots (\text{eq. 2})$$

Where, pt is tapped density, M is weight of the powder, Vt is tapped volume.

c) Angle of repose:

Angle of Repose was determined using funnel method. The blend was poured through a funnel that can be raised vertically until a maximum pile height (h) was obtained. Radius of the pile (r) was measured and angle of repose (θ) was calculated using the form.

$$\text{Angle of repose } (\theta) = \tan^{-1} \left(\frac{h}{r} \right) \dots (\text{eq. 3})$$

Where, θ is Angle of Repose, h is height of pile, r is radius of pile.

Table No. 2: Angle of repose

Angle of repose (°)	Type of flow
<25	Excellent
25-30	Good
30-40	Passable
>40	Poor

d) Compressibility Index:

The simplest way for measurement of free flow of powder is compressibility, an indication of the ease with which a material can be induced to flow is given by compressibility index (I) which is calculated as follows

$$\text{Compressibility Index} = 100 \times \left(\frac{\rho_{\text{tapped}} - \rho_{\text{bulk}}}{\rho_{\text{tapped}}} \right) \dots (\text{eq. 4})$$

Where; I is compressibility index, ρ_t is Tapped Density, ρ_b is Bulk Density.

Table No. 3: Compressibility index

Carr's index (%)	Type of flow
<12	Excellent
12-17	Good
18-21	Fair to passable
22-32	Poor
33-38	Very poor
>40	Extremely poor

Table No. 5: Determination of purity of powder

S.no.	Tests for Powder	Observation
1	Test for steroids: Libermann – burchard test	Absent
2	Test for saponins : Foam test	Absent
3	Test for Carbohydrates: Molisch test, Barfoed's test, Benedicts test	Present
4	Test for Flavonoids: Shinoda test, Zinc/HCl reduction test	Absent
5	Test for Tannins/ Phenols: Ferric chloride test, Gelatin test	Absent
6	Test for Glycosides: Killer	

Solubility Profile:

The solubility profile of the powder was found as shown in Table 5. The powders were insoluble in acetone, alcohol, ether, chloroform. It was found to form a gel in hot water.

Table No. 6: Solubility Index

S.no.	Solvents	Observations
1	Acetone	Insoluble
2	Methanol	Insoluble
3	Ethanol	Insoluble
4	Chloroform	Insoluble
5	Hot Water	Forming a gel
6	Cold water	Insoluble

e) Hausner's Ratio:

Hausner ratio (HR) is an indirect index of ease of powder flow. It is calculated by the following formula.

$$\text{HR} = \rho_t / \rho_b \dots (\text{eq. 5})$$

Where HR is Hausner's ratio, ρ_t is tapped density, ρ_b is bulk density.

Lower Hausner's ratio (< 1.25) indicates better flow properties than higher ones (> 1.25).^[7]

RESULT AND DISCUSSION

Organoleptic Evaluation:

The polysaccharide was characterized by various organoleptic properties such as color, odor, taste, shape, touch and texture and shown in Table 3.

Table No. 4: Organoleptic evaluation of the mucilage powder

S. No.	Property	Observation
1	Color	Off white
2	Odor	Odorless
3	Taste	Tasteless
4	Shape	Irregular
5	Touch and texture	Hard and rough

Phytochemical screening of the powder:

The basic Phytochemical screening tests for carbohydrates, alkaloids, steroids, flavonoids saponins, tannins and phenols were carried out and shown in Table.4. The tests indicated the absence of alkaloids, steroids, flavonoids, saponins, tannins and phenols. Only carbohydrates were found to be present.

Determination of Swelling Index:

Swelling index of powder sample in distilled water was found to be 60. High swelling index indicated that the starch has excellent water uptake capacity.

Swelling index = 60

Moisture Absorption:

Moisture absorption of powder sample was determined as per procedure adopted Hygroscopicity influences the packaging, storage of the products.

Moisture absorption = 2.45

Determination of pH of Powder:

The powder sample was subjected to determine the pH in digital pH meter.

pH = 6.2

Determination of Melting Point:

Melting point of powder sample was determined by capillary fusion method. The melting point was recorded 37°C.

Loss of Drying:

The powder sample was subjected for determining the LOD in hot air oven.

Loss on drying = 0.25%

Thermal Stability:

Thermal stability study was established and the barley starch withstands to temperature up to 120°C.

Flow Properties of Black gram Extract:

The derived properties such as bulk density, tapped density, compressibility index, hausner's ratio and angle of repose which depend mainly on particle size distribution, particle shape and tendency of the particles to adhere together results shown in table no.6. The values of bulk density, compressibility index and Hausner's ratio infer that the Barley extract has fine flow properties and compressibility.

Table No. 7: Characterization of Barley starch

S.no	Properties	Observation
1	Bulk density (gm/cm ³)	0.43
2	Tapped density (gm/cm ³)	0.52
3	Compressibility index (%)	17.30
4	Angle of repose (°)	35.6
5	Hausner's Ratio (HR)	1.21

CONCLUSION

The starch from black gram has been extracted from methods mentioned above.. This extracted powder was studied as for the identification, characterization and flow properties which are helpful in pharmaceutical industry. Carbohydrates

were present in the powder, which is insoluble in Acetone, Ethanol and in Chloroform. The powder was forming the gel in hot water but in cold water it was poorly soluble. The pH was found to be 6.2, swelling index was 60, Melting point was 37°C, Moisture absorption was 2.35, Loss on drying 0.25%, and thermal stability was calculated as 120°C. The bulk density was found to 0.43, Tapped density was found to 0.52, angle of repose was found to be 35.6 and the compressibility index was found to 17.30, hausner's ratio was found to be 1.21 respectively. The Black gram extract was found to possess a fine flow properties hence can be used as pharmaceutical excipient.

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